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The Germicidal Action of Ultra-Violet Radiation, and its Correlation with Selective Absorption.

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[Plate 3.]

A new method is here described which enables us to say definitely what portion of the ultra-violet spectrum is especially effective in germicidal action and the wave-length of the radiation at which such action practically ceases. Briefly the method consists of inoculating a gelatine* plate with micro-organisms instead of sensitising it with a silver salt. We find that when a spectrum is formed on this it produces what may be called an image, where germicidal action occurs, and this image may be rendered visible by a process of incubation, which encourages a copious growth of those organisms which have not been affected by the radiation, whereas the affected parts remain practically transparent. Such an exposed and incubated plate can be used as an ordinary negative for producing positive contact prints or, equally well, may be photographed by light reflected and scattered from the bacterial surface.

The study of the action of radiation, visible and otherwise, upon micro-organisms is not a new one. The period 1894–6, associated with the work

* For convenience, an agar plate was used in most of the experiments.

of Marshall Ward,* Wesbrook,† and D'Arcy and Hardy,‡ conferred upon such investigations an exactness which had been previously entirely lacking, both as regards knowledge of the most effective germicidal portion of the visible rays, and also with regard to the essential chemical processes associated with such action.

The present investigation deals with one particular region of ultra-violet radiation, which we find has much greater germicidal action than any part of the visible spectrum, and an attempt is made to correlate such germicidal action with the physical phenomenon of selective absorption.

The quartz spectrometer (Design C), made by Messrs. Hilger, is so constructed that photographs may be obtained ranging from wave-lengths 7000–2100 Å.U.;§ this region of the spectrum is spread over a length of about 19 cm. (it varies slightly with each instrument).

The radiation emitted by an arc of pure tungsten, when focussed on the slit of the instrument and received on the photographic plate, is seen to consist of a series of bright lines very closely packed together extending as far as 2100 Å.U. Thus it is a very suitable source of intense ultra-violet radiation over a wide range.

In order to determine what part of this radiation exerts germicidal action, the following procedure was employed: A glass plate, similar to the photographic plate used in this instrument, was coated with a thin layer of nutrient agar; then over this surface a thin layer of a living bacterial emulsion (*Staphylococcus pyogenes aureus*) was painted. The plate thus inoculated was then placed in the carrier of the instrument, and the shutter opened to allow the radiation from the arc to fall upon a narrow strip of the bacterial film. After an appropriate exposure the plate was removed from the spectrometer, and then incubated at 37° C. for 48 hours in order to see, by the resulting growth, what effect had been produced upon the organisms by the various constituents of the beam.

Fig. 1 shows the germicidal effect of the rays upon *Staphylococcus pyogenes aureus* for three different times of exposure of the organisms, viz., 6, 12, and 24 minutes. The radiation utilised, as shown by its action on a photographic plate, extended right across the bacterial film; the germicidal action is, however, restricted to a region which ranges from a wave-length of 2940 to about 2380 Å.U. The black lines indicate the bactericidal action. The illustration was obtained by photographing the bacterial film by means of

* Marshall Ward, 'Phil. Trans.,' B, 1894, p. 961.

† Wesbrook, 'Journ. Path. and Bact.,' vol. 3, p. 70 (1896).

‡ D'Arcy and Hardy, 'Journ. Physiology,' vol. 17, No. 5, p. 390 (1894).

§ Å.U. signifies Ångström units, the standard by which wave-lengths are measured.

transmitted light. From this negative a transparency was made, and this was employed for the print.

Photographs taken with long exposures always show the presence of scattered radiation, and it is interesting to observe that the organisms appear to be stimulated in their growth in the region just between two portions which have been exposed to the full radiation. The increase of bacterial growth was confirmed microscopically, but it is not necessarily due to the scattered radiation. This stimulation is only seen satisfactorily in the original.

The effect of prolonging the exposure from 6 to 24 minutes is to increase somewhat the range of lethal action, but only to a comparatively slight extent. A more prolonged exposure brings out prominently the region of the spectrum at which germicidal action practically stops. In order to increase the amount of radiation reaching the bacterial film, the slit was widened; this had the effect of causing the lines of the spectrum to overlap sufficiently to form a practically continuous band of radiation.

The procedure followed was otherwise identical with that which has just been described; the bacterial film was first exposed to the whole range of spectral lines, then incubated, to see what effect had been produced upon the organisms by the various constituents of the beam.

The central strip of fig. 2 shows the result of a prolonged exposure (about 3½ hours) of the organisms to the tungsten radiation. It will be seen that germicidal action, indicated by the black region, occurs throughout a region of wave-lengths extending from about 2960 to 2150 Å.U. The most striking feature is the sharp line of demarcation occurring in the region of the former wave-length. Ether vibrations of wave-length 2960 Å.U. have a marked germicidal action; an increase of 1 or 2 per per cent. in this wave-length is, practically speaking, sufficient to bring one to a region of the spectrum devoid of such germicidal action.

A control experiment was made to decide whether the radiation had any effect upon the agar. A portion of it was irradiated, and then inoculated with a suspension of an agar culture of *Staphylococcus pyogenes aureus*, which had been washed three times by centrifuging with sterile 0·8 per cent. sodium chloride solution. No difference was detected in the density of growth of the organisms over the irradiated and the non-irradiated portions of the agar.

The existence of this sharp line of demarcation strongly suggested that selective absorption was playing some part. This was put to the test by examining the absorption spectrum of a suspension of the organisms which were used in the experiment just described. A small quartz vessel was filled with the bacterial emulsion and placed in front of the slit of the

spectrometer; a series of photographs was then taken of the radiation transmitted through the emulsion. The bottom strip (fig. 2) shows that the region of wave-lengths absorbed by the bacterial emulsion corresponds almost exactly with that portion of the spectrum which has marked germicidal action. We shall return to this point later on.

The Germicidal Action upon Various Organisms.

The method which has been described to determine what portion of the ultra-violet spectrum is responsible for germicidal action is well adapted for comparative tests of the effects of these rays upon different organisms.

(a) *Bacillus coli communis and Bacillus typhosus.*—To determine the respective ranges of susceptibility of these organisms, emulsions from agar cultures were made and spread over a surface of agar, which took the place of the photographic film, as already described. A central strip, about 3 cm. wide, running the whole length of the plate, was separated from the rest of the agar by two glass strips a few millimetres in width. The central strip was painted over with the emulsion of one of the organisms, and the rest of the agar with that of the other organism. The plate was then placed in position in the camera, and the length of the slit adjusted so that the radiation from the arc illuminated both surfaces over which the organisms were spread, one of the glass strips lying exactly along the middle of the exposed area. In this way the two organisms were exposed to radiation of identical character for the same time; after a suitable exposure, the camera attachment was racked down, so that the second glass strip was in the centre of the exposed area, and another exposure made. The agar plate was removed and incubated overnight; the resulting growth on the plate showed which organism had been affected by a greater range of the rays.

A series of observations, ranging from 5–10–20–30 minutes, showed that a lethal effect upon *B. typhosus* was obtained over a wider range than with *B. coli;* this difference in range of susceptibility was small, but quite definite for each of the exposures: for an exposure of five minutes, a lethal effect was obtained on the *B. coli* over a range of wave-lengths 2960–2450 Å.U.; for the *B. typhosus* the range was 2960–2400 Å.U.; when the exposure was lengthened to 30 minutes, the lethal effect on *B. coli* ranged from 2960 to 2200 Å.U.; and that of the *B. typhosus* from 3000 to 2100 Å.U. Such small differences could hardly serve as a sure method of differentiation of these organisms.

(b) *Acid-fast Bacillus (Timothy Grass B) and the Meningococcus.*—The ranges of susceptibility of these organisms to the rays in question was compared directly with staphylococcus by the method detailed above. (In

the experiments in which the meningococcus was compared, "trypagar" was employed as the medium.) After suitable exposures the agar plates were incubated, and the range of wave-lengths over which a lethal action was subsequently observed was measured for the different organisms. The meningococcus was found to be susceptible over a slightly greater range than the staphylococcus; exposure of these two organisms to the same radiation for the same time (20 minutes) resulted in a lethal action upon the meningococcus over a range 2960–2240, the range for staphylococcus being 2960–2320 Å.U.

In the case of the acid-fast bacillus, the result was reversed, the staphylococcus being slightly more susceptible than the acid-fast bacillus.

The Connection between Germicidal Action and Selective Absorption.

The photograph which serves to illustrate the selective absorption of the bacterial emulsion was obtained with an exposure of two minutes. A single observation is obviously not sufficient to prove that the region beyond a wave-length of 2960 Å.U. is selectively absorbed. Therefore a series of photographs was taken in which the exposure ranged from five seconds to five minutes, and the fact that practically identical records resulted, except in the respective densities of the spectral lines transmitted through the bacterial emulsion, may be taken as evidence that wave-lengths shorter than 2960 Å.U. are actually absorbed in a selective manner. By increasing the exposures to as much as 20 minutes, only a few additional lines made their appearance beyond the line of demarcation in question.

It seems therefore that the conclusion may justifiably be drawn that ultra-violet radiation between wave-lengths 2960 and 2100 Å.U. is germicidal to bacteria, and that rays over this range of wave-length are also particularly absorbed by the substances of which such bacteria are composed. We have found experimentally that such substances as human serum and egg albumen also have a well marked absorption band for wave-lengths ranging from 3180 to 2100 Å.U. (and also possibly beyond, but this we have not investigated). If further we recall the fact that human skin in a layer as thin as 1/10 mm. is practically opaque to radiation over a very similar range of wave-lengths, then we may look upon this region as one for which protoplasm has a particular power of absorption. The high degree of correlation between the germicidal action of a particular portion of the ultra-violet radiation with an enhanced degree of absorption of such radiation by the organisms, which appears to have been clearly established, does not, of course, explain the bactericidal effect, but the enquiry now takes on a physico-chemical aspect as well as a purely biological one. Attention may be called

to the correspondence between our result and that found by Grotthus in 1818* for photo-chemical action, which he expressed in the law "that only those rays which are absorbed can produce chemical change."

The Bearing of the Observations on the Clinical Uses of Ultra-Violet Radiation.

The preceding data indicate that, from the clinical point of view, there are two distinct regions of ultra-violet radiation :—

Group 1.—A portion which begins where vision fails, namely 3800, and extends to 2960 Å.U. These rays have no marked germicidal action; they are capable, however, of penetrating a considerable thickness of human skin.

Group 2.—A portion which extends from 2960 to nearly 2100 Å.U. These rays have very marked germicidal action, the region of maximum effectiveness being between 2800 and 2540 Å.U. The penetrating power of these rays is, however, very small; they are completely absorbed by as little as 1/10 mm. of human skin.

It remains to consider to what degree these two regions of the ultra-violet radiation contribute to the beneficial effects resulting from the clinical use of these rays.

If a powerful source of ultra-violet radiation be directed upon an infected wound, the result of an adequate exposure will be that the pathogenic organisms on the surface will be directly killed; it is, of course, not determined what action these particular rays may themselves exert on the protective mechanism of the living tissues. Organisms at a depth cannot be killed directly, for the germicidal rays do not reach them. The passage through the tissues of rays constituting Group I, together with the luminous portion of the rays, may not, however, be without effect upon them. It is well to bear in mind the possibility of the luminous rays having definite physiological effects, for it must be remembered that such rays penetrate to a greater depth of the tissues than does even Group I of the ultra-violet rays.

It has been stated by several writers that deep-seated conditions have been benefited by exposure to some strong source of visible and ultra-violet radiation. It remains to be found to what extent the different parts of these two octaves of radiation are concerned in such clinical results, although, as has been already stated, there is no evidence that deeply situated organisms can be affected by those ultra-violet radiations which are known to possess powerful bactericidal action.

We have pleasure in recording our thanks to the British Thomson-Houston Company for their gift of the tungsten used in this investigation.

* See Gilbert's 'Ann. d. Phys.,' vol. 61, p. 50 (1819).

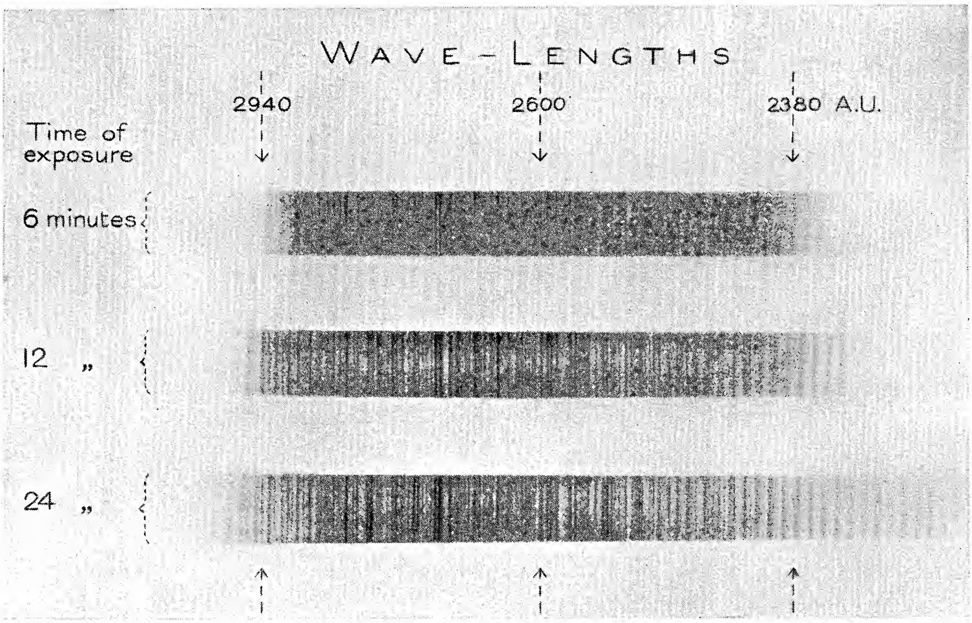

Wave-Lengths
2940
2600
2380 A.U.
Time of exposure
6 minutes
12 „
24 „

Fig. 1.

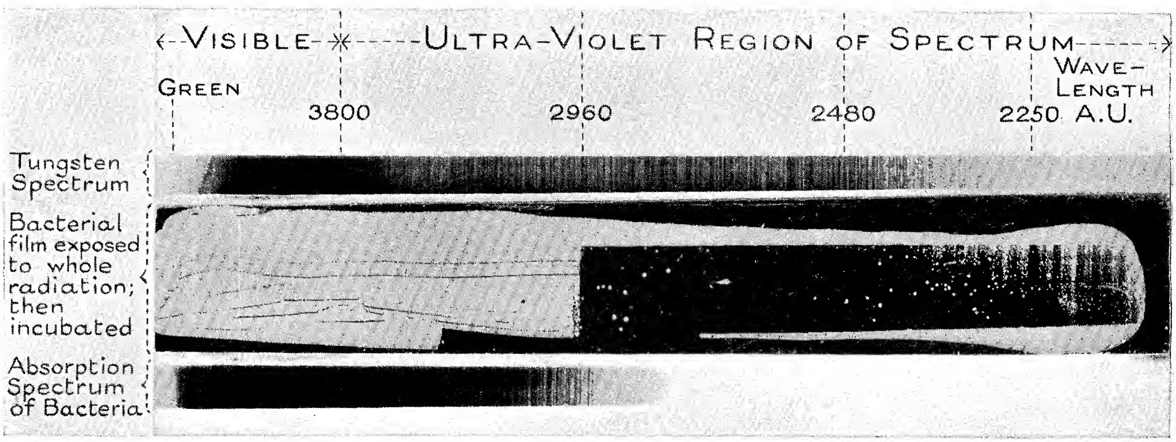
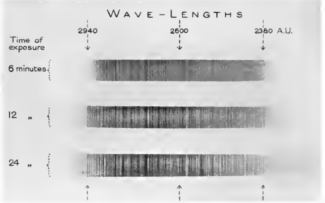
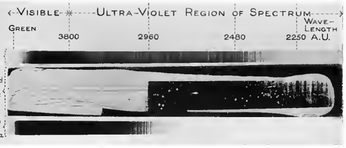

←Visible→←Ultra-Violet Region of Spectrum→
Green
3800
2960
2480
2250 A.U.
Wave-Length
Tungsten Spectrum
Bacterial film exposed to whole radiation; then incubated
Absorption Spectrum of Bacteria

Fig. 2.



WAVE-LENGTHS
2940
2600
2380 A.U.
Time of exposure
6 minutes
12 „
24 „

FIG. 1.



←VISIBLE→←ULTRA-VIOLET REGION OF SPECTRUM→
WAVE-LENGTH
GREEN
3800
2960
2480
2250 A.U.
Tungsten Spectrum
Bacterial film exposed to whole radiation; then incubated
Absorption Spectrum of Bacteria

FIG. 2.